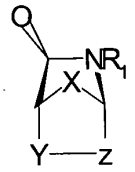
PROCESS FOR THE PREPARATION OF OPTICALLY ACTIVE AZABICYCLO HEPTANONE DERIVATIVES

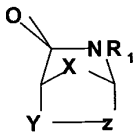
Field of the invention

The present invention relates to a process for the preparation of an optically active azabicyclo heptanone derivative of general formula III



Formula III

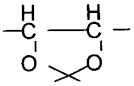
wherein R_1=H, X=CH_2 Y-Z = -CH=CH- from racemic lactam of formula (I)

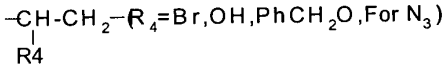


Formula I

Wherein R_1 is H or COR_2 (R_2 is C_{1-4} alkyl, C_{1-4} alkoxy, aryl or aryloxy)

X is O or CHR_3 (R_3 is F, OH, Br or H)

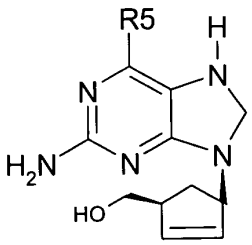
Y-Z=-CH=CH-,CH_2-CH_2, 



The present invention also relates to a process for the preparation of an optically active azabicyclo heptanone derivative of general formula III wherein R_1=H, X = CH_2, Y-Z = CH = CH_2 useful as an intermediate in the synthesis of antiviral agents.

Background of the invention

Carbocyclic analogues of purines are known as antiviral and anti neoplastic agents. For example the compound of formula (II) is described as having potent activity against human immunodeficiency virus (HIV) and Hepatitis B virus (HBV) (EP 0434450).



Formula - II

R_5 =Cyclopropylamino, =N-Cyclopropyl, N-Methylamine

Prior art discloses the preparation of 9-substituted-2-amino purines starting from a pyrimidine compound, coupling with enantiomerically pure sugar/carbocyclic analogues residue and cyclization to form the imidazole ring followed by introduction of suitable 6-substituent (PCT/GB95/00225). Carbovir and known analogues are prepared from the known -lactam(Vince lactam)2-azabicyclo[2,2,1]hept-5-en-3-one (Formula I) wherein X is $-CH_2$, -Y-Z- is –CH=CH- and R_1 is H.

Prior art indicates that the final product or any intermediate or starting material may be resolved by known methods or the racemic mixture of the product may be enzymatically converted to chirally pure compound. The -lactam can be prepared by reacting cyclopentadiene with tosylcyanide,(Vince J. Org. Chem. 1978, 43, 2311).

There are several synthetic pathways where chemical resolution into the enantiomer has been effected but the enzymatic resolution of -lactam will be the most economical commercial process. γ-lactamase methodology has been reported based on enantio complementary biotransformation. Enzymatic resolution of bicyclic lactam using whole cell cultures ENZA1 and ENZA2 has been reported to give both the optical forms of lactam (S.V. Teylor, J.C.S. Chem. Comm., 1121, 1990, Tet. Assy., 4, 1117-1128). The detailed process has been described in patent (EP 0424064). The racemic lactam was treated with ENZA-1/2 cell free extract at 30°C with shaking for 14 days. The crude (+)/ (-) lactam was isolated by extraction with dichloromethane purified by column chromatography on silica gel. The (+) lactam obtained with 88% ee and (-) lactam 98% ee.

Enzymatic resolution of N-Acyl bicyclic lactam using acylase has been described in patent (PCT/EP99/04814) in 31% yield with 98% ee. The conversion of the optically active N-Acetyl-lactam to(+) /(-) lactam is tedious.

The prior art methods to the cyclopentane moiety of carbocyclic nucleosides starting from non-carbohydrate synthons or readily available meso compounds generally involve a number of steps, are often difficult to perform and provide poor yields making practical scale-up of these processes difficult and uneconomical.

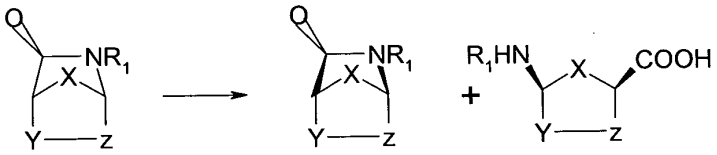
Objects of the invention

The main object of the present invention is to provide a process for the preparation of an optically active azabicyclo heptanone derivative which obviates the drawbacks of the present invention and use cheaper and easily available microbial whole cell enzyme.

It is another object of the invention to provide a process for the preparation of (-) 2-Azabicyclo[2,2,1]-hept-5-ene-3-one formula (III) which is economical and efficient.

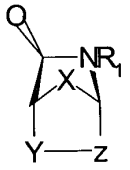
Summary of the invention

The present invention provides a process for preparation of optically active azabicyclo heptanone derivatives using lactamases that will react with racemic -lactam of formula (I) to give a single enantiomer of lactam (III) and the corresponding ring opened compound of formula (IV) in an enantiomerically pure form.



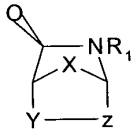
I III(-) IV(+)

Accordingly, the present invention provides a process for the preparation of (-)2-Azabicyclo[2,2,1]-hept-5-ene-3-one formula(III) wherein R_1=H, X=CH_2 Y-Z = -CH=CH-



Formula III

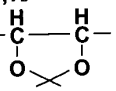
which comprises reacting a racemic mixture of compound of formula (I)

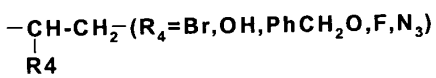


Formula I

Wherein R_1=H, COR_2 (R_2=C_{1-4} alkyl,C_{1-4} alkoxy,aryl,aryloxy)

X=O,CHR_3 (R_3=F,OH,Br,H)

Y-Z=-CH=CH-,CH_2-CH_2, 



(R_4=Br,OH,$PhCH_2O$,F,N_3)

with enzyme or whole cells in a buffer containing organic solvent at temperature ranging between 25-30°C for a period ranging from 14 to 24 hr. extracting the mixture in an organic solvent, separating the organic layer and removing the solvent to obtain the product.

In one embodiment of the invention the microorganisms or enzymes used are selected from the *Bacillus, Klyuvera* and *Eschericha*.

In another embodiment of the invention the whole cell is obtained from growing culture *Klyuvera Citrophila* ATCC No.21285 in a conventional culture medium.

In another embodiment of the invention, the cell extract or enzyme used comprises enzyme or cell extract from *Klyuvera sp.* (ATCC No.21285).

In another embodiment of the invention the buffer used is selected from the group consisting of phosphate buffer (0.05 M – 0.1 M, 6-8 pH), citrate buffer (0.05 M – 0.1 M 6-7.5 pH) and Trisbuffer (0.05M- 0.2M, 7-8 pH).

In another embodiment of the invention the buffer used comprises phosphate buffer (0.2 M, 7.4 pH).

In another embodiment of the invention the organic solvent used for the reaction along with buffer is selected from the group consisting of alcohols, alkyl acetates, ketones and sulfoxides.

In a further embodiment of the invention, the organic solvent is selected from the group consisting of methanol, ethanol, butanol, ethyl acetate, acetone, dimethyl sulfoxide and dimethylformamide.

In a further embodiment of the invention, the organic solvent comprises acetone.

In another embodiment of the invention the percent of organic solvent used for the reaction along with buffer is in the range of 5% to 50% (v/v).

In another embodiment of the invention the percent of organic solvent used for the reaction along with buffer comprises is 10 %(v/v).

In another embodiment of the invention the solvent used for extraction comprises a chlorinated solvent selected from the group consisting of chloroform, ethylene dichloride, methylene dichloride and an alkyl acetate.

In another embodiment of the invention, the alkyl acetate used as the solvent for extraction comprises ethyl acetate.

In another embodiment of the invention the solvent used for extraction comprises methylene chloride.

In a feature of the invention the chemical yield (-) 2-Azabicyclo[2,2,1]-hept-5-ene-3-one is 39.3% and optical purity is 98%.

Detailed description of the invention

The process of the present invention is highly efficient and maximizes cost effectiveness by fast resolution to provide (-) lactam enantiomer, an important starting material for the production of the anti HIV agent (-) Carbovir and Abacavir.

The process of the present invention is described herein below with references to following examples, which are illustrative only and should not be construed to limit the scope of the present invention in any manner.

Example 1

This example describes general procedure for cell biomass preparation (whole cell)/enzyme pre-inoculum (5-10 ml) was prepared by growing microorganism in a medium containing yeast extract (0.5%), peptone (1%), sodium chloride (0.2%), sodium glutamate (0.5%) and phenyl acetic acid (15 mM) at pH 7.2-7.3 for 24 hr with shaking at 150rpm. This was subsequently transferred to 1 Lt. flask containing 300 ml above mentioned growth medium and incubated at 28-30°C for 24 hours on rotary shakers (150 rpm). The grown cells were separated by centrifuge washed with phosphate buffer pH 6.8 and biomass was used for the reaction.

Example 2

General procedure for enantioselectsive hydrolysis of (±)-2-azabicyclo (2,2,1) hept-5-en-3-one, (Vince's lactam) (1).

0.1 g (0.00092 mole parts) of (±)-2-azabicyclo[2,2,1]-hept-5-en-3-one (1) was suspended in phosphate buffer (5 parts) and 50 mg of wet cell mass of culture (ATCC No.21285) was added and kept stirring 72 hr. The cell mass was removed by filtering through celite and the filtrate was extracted with dichloromethane (5 x 10 parts). Concentration of solvent gave optically active III in 31.8% chemical yield, and 58.2% ee.

Example 3

General procedure for enantioselective hydrolysis of (±)-2-azabicyclo(2,2,1)hept-5-en-3-one, using cell mass from culture (ATCC No.21285). 0.1 g (0.00092 mole parts) of (±) was suspended in phosphate buffer (5 parts) and different amount of cell mass (as indicated in Table 1) was added and kept stirring 24 hr. The cell mass was removed by filtering

through celite and the filtrate was extracted with dichloromethane (5x10 parts). Concentration of solvent gave optically active III. The results are summarized in Table 1.

Table 1

Sr.No.	Cells Wet/Wt.%	Chemical Yield	Ratio R:S	ee %
1.	5	39.1	46.36 : 53.64	7.29
2.	10	33.2	45.15 : 54.85	9.71
3.	20	31.4	36.70 : 63.30	26.61
4.	30	33.0	27.43 : 72.57	45.14
5.	40	34.2	25.71 : 74.29	48.58
6.	50	30.5	21.23 : 78.77	57.55

Example 4

General procedure for enantioselective hydrolysis of (±)2-azabicyclo(2,2,1)hept-5-en-3-one, using cell mass from culture (ATCC No.21285). 0.2 g (0.00184 mole parts) of (±) was suspended in phosphate buffer and organic solvent,(as indicated in Table 2) 10 parts. 0.1 gm of wet cell mass was added and kept stirring 24 hrs. The cell mass was removed by filtering through celite and the filtrate was extracted with dichloromethane (5 x 10 parts). Concentration of solvent gave optically active III. The results are summarized in Table 2.

Table 2

Sr.No.	Organic Solvent	Chemical Yield	Ratio R:S	ee %
1.	Ethyl acetate	27.1	13.39 : 86.61	73.22
2.	Methanol	25.5	14.90 : 85.10	70.20
3.	Ethanol	33.1	9.27 : 90. 73	81.46
4.	Acetone	44.2	9.69 : 90.31	80.62
5.	Dimethyl sulfoxide	42.1	36.10 : 63.90	27.80

The ratio of phosphate buffer (0.2M, pH 7.4) to organic solvent is (9:1).

Example 5

General procedure for enantioselective hydrolysis of (±)2-azabicyclo(2,2,1)hept-5-en-3-one, using cell mass from culture (ATCC No.21285). 0.2 g (0.00184 mole parts) of (±) was suspended in phosphate buffer and acetone (as indicated in Table 2) 10 parts. 0.1 gm of wet cell mass was added and kept stirring 24 hrs. The cell mass was removed by filtering through celite and the filtrate was extracted with dichloromethane (5 x 10 parts). Concentration of solvent gave optically active III. The results of different proportions of acetone are summarized in Table 3.

Table 3

Sr.No.	Buffer : Acetone(v/v)	Chemical Yield	Ratio R:S	ee %
1.	9.5 : 0.5	40.8	4.58 : 95.85	90.85
2.	9.0 : 1.0	41.2	9.69 : 90.31	80.62
3.	8.0 : 2.0	41.3	12.34 : 87.66	75.33
4.	5.0 : 5.0	Slow reaction	-	-

Example 6

General procedure for enantioselective hydrolysis of (±)2-azabicyclo(2,2,1)hept-5-en-3-one, using cell mass from culture (ATCC No.21285). 10.0 g (0.918 mole parts) of (±) was suspended in mixture of 475 parts of phosphate buffer and 25 parts of acetone in Lt. flask. Cell mass (wet. Weight ~ 5 parts) was added and the reaction mixture was stirred at room temperature (28±1). After completion of the reaction (monitored by chiral HPLC) the reaction mixture was centrifuged in order to remove cell mass and supernatent liquid was extracted using continuous extractor by dichloromethane. On evaporation of solvent under reduced pressure (1S, 4R) azabicyclo(2,2,1)hept-5-en-3-one III (3.93 gm) was obtained. Crystallisation with dichloromethane : ether mixture gave a product of 98% optical purity.